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(71) Applicant: **THE PROCTER & GAMBLE COMPANY**  
301 East Sixth Street  
Cincinnati Ohio 45202(US)

(72) Inventor: **Uchtman, Vernon Albert**  
854 Carol Lane  
Cincinnati Ohio 45218(US)

(74) Representative: **Brooks, Maxim Courtney et al,**  
Procter & Gamble (NTC) Limited Whitley Road  
Longbenton  
Newcastle-upon-Tyne NE12 9TS(GB)

(54) **Kit for use in the treatment of osteoporosis.**

(57) **A kit for use in the treatment of osteoporosis is disclosed. The kit comprises a bone cell activating compound, a bone resorption inhibiting polyphosphonate, and a nutrient supplement or placebo, for sequential administration.**

0162510

KIT FOR USE IN THE TREATMENT OF OSTEOPOROSIS  
Vernon A. Uchtman

TECHNICAL FIELD

5       The present invention relates to a kit for use in a regimen for treatment or prevention of osteoporosis. Specifically, the present invention relates to a kit for use in a regimen whereby a bone cell activating compound, a bone resorption inhibiting polyphosphonate, and a placebo or a nutrient supplement is administered sequentially to a subject afflicted with or at risk to  
10       osteoporosis.

      Osteoporosis is the most common form of metabolic bone disease. Although it may occur secondary to a number of underlying diseases, 90% of all cases appear to be idiopathic.

15       Idiopathic osteoporosis is most commonly observed in postmenopausal women (postmenopausal osteoporosis) but it may also occur in elderly males and females (senile osteoporosis) or occasionally in younger individuals of both sexes. The disease which develops in postmenopausal women is characterized primarily by fractures of the wrist and spine, while femoral fractures seem to  
20       be the dominant feature of senile osteoporosis.

      The fractures which occur in the various forms of osteoporosis are caused primarily by a gradual loss of bone which eventually reaches the point of mechanical failure. The physical nature of the bone which remains also seems to be compromised  
25       but the role which this plays in the loss of bone strength is unclear.

      The mechanism by which bone is lost in osteoporotics is believed to involve the process by which the skeleton renews itself. This process has been termed bone remodeling. It occurs  
30       in a series of discrete pockets of activity. These pockets appear spontaneously within the bone matrix on a given bone surface as a site of bone resorption. There is apparently an activation of precursor cells within these pockets to form osteoclasts (bone dissolving or resorbing cells) which, in turn, resorb a portion of  
35       bone of generally constant dimensions. This process is followed by the appearance of osteoblasts (bone forming cells) which then refill the cavity left by the osteoclasts with new bone.

In a healthy adult subject, the rate at which osteoclasts and osteoblasts are formed is such that bone formation and bone resorption are in balance. However, in osteoporotics an imbalance in the bone remodeling process develops which allows bone to be lost at a rate faster than it is being made. Although this imbalance occurs to some extent in most individuals as they age, it is much more severe and occurs at a younger age in osteoporotics, particularly those who develop the postmenopausal form of the condition.

There have been many attempts to treat osteoporosis with a variety of pharmacologic agents with the goal being to either slow further bone loss or to produce a net gain in bone mass. It appears as though there are agents available which will slow further bone loss in osteoporotics but agents or methods of treatment which will result in the replacement of bone which has already been lost have been very elusive. The reason for this probably lies in the tight coupling characteristics of bone remodeling. Agents or methods of treatment which stimulate or suppress one phase of the cycle (either resorption or formation) tend to have a similar effect on the opposing process. Therefore most attempts to treat osteoporosis have resulted in no more than a transient change and when the opposing process is stimulated or suppressed, the change is then negated.

Using a different approach, it has been attempted to induce bone activation by continuous administration of inorganic phosphate and to separately inhibit bone resorption by intermittent administration of calcitonin. This method has been shown to result in net bone formation in patients with post-menopausal osteoporosis. Furthermore, a theoretical model has been proposed which suggests that it may be possible to synchronize bone cell activity and metabolism by administering bone activating agents. Once synchronized, it should then be possible to limit the resorption by administering a bone resorption inhibiting agent during the natural life of the resorption phase of the bone remodeling units. The model does not address the problem of bone formation inhibition which is typically associated with the administration of a

bone resorption inhibiting agent.

It is therefore an object of the present invention to provide a kit for treating osteoporosis which does not require a prolonged administration of pharmacologic agents, and which does not result  
5 in a significant inhibition of bone formation.

#### BACKGROUND OF THE INVENTION

U.S. Patent 4,230,700, issued October 28, 1980 to Francis, discloses the conjoint administration of certain polyphosphonate compounds, in particular diphosphonates, and vitamin D-like  
10 anti-rachitic compounds for inhibition of the anomalous mobilization of calcium phosphates in animal tissue. U.S. Patent 4,330,537, issued May 18, 1982 to Francis, claims the compositions used in the methods of the '700 patent. The patents specify that the administration of the phosphonate and the vitamin D-like com-  
15 pound be conjoint; moreover, the vitamin D-like compounds (unlike certain vitamin D metabolites) do not qualify as bone cell activating compounds.

Rasmussen et al., "Effect of Combined Therapy with Phosphate and Calcitonin on Bone Volume in Osteoporosis", Metabolic  
20 Bone Disease and Related Research, 2, 107 (1980), discloses a treatment regimen consisting of continuous administration of inorganic phosphate and intermittent administration of calcitonin. A net bone formation was observed.

Frost, "Treatment of Osteoporosis by Manipulation of Coherent Bone Cell Populations", Clinical Orthopedics and Related  
25 Research, 143, 227 (1979), discloses a theoretical model which suggests that it should be possible to synchronize the activity and metabolism of bone cells by administering bone cell activating agents. Once the cells have been synchronized, their resorption  
30 activity could be effectively inhibited by administration of a bone resorption inhibiting agent. The model requires that the bone resorption inhibiting agent be administered throughout the bone resorption phase of the bone remodeling unit. Furthermore, the  
35 model suggests that administration of a high dose of the bone resorption inhibiting agent is desirable because the bone resorption should be inhibited as much as possible. The model

assumes that bone formation inhibition does not take place, because no bone resorption inhibiting agent is administered during the bone formation phase of the bone remodeling unit.

#### SUMMARY OF THE INVENTION

- 5       The present invention relates to a kit for use in a regimen for treatment or prevention of osteoporosis, said regimen comprising sequential administration of a bone cell activating compound, a bone resorption inhibiting polyphosphonate, and a placebo or a nutrient supplement, said kit containing the following components:
- 10   (a) from 1 to about 5 daily doses of a bone cell activating amount each of a bone cell activating compound; (b) from about 10 to about 20 daily doses of from about 0.25 X LED to about 3.3 x LED each of a bone resorption inhibiting polyphosphonate; and
- 15   (c) from about 30 to about 100 daily doses of a placebo or a nutrient supplement; and a means for having the components arranged in a way as to facilitate compliance with the regimen.

#### DETAILED DESCRIPTION OF THE INVENTION

- The kit of the present invention is designed for use in a treatment regimen which consists of one or more cycles, whereby
- 20   each cycle consists of a bone activating period, a bone resorption inhibition period and a rest period. During the bone activating period, bone cells are induced into a synchronized metabolism. During the bone resorption inhibition period, the bone resorption which naturally follows the activation is limited to a minimum by
- 25   administration of a bone resorption inhibiting polyphosphonate. The rest period allows for natural bone formation to occur. It is desirable to administer food supplements, like calcium or vitamin D, during the rest period, so as to ensure optimum conditions for bone formation. If the administration of food supplements is
- 30   deemed unnecessary, e.g., where the patient is on a fully controlled diet, the kit must contain daily doses of a placebo, to be taken during the rest period, so as to ensure a strict compliance with the regimen.

- The preferred mode of administration is orally, but other
- 35   modes of administration include, without limitation, intramuscular, intravenous, intraperitoneal, and subcutaneous administration, as

well as topical application. All compounds described herein are administered orally, except where specified otherwise.

By "subject afflicted with or at risk to osteoporosis" as used herein is meant a subject diagnosed as suffering from one or more of the various forms of osteoporosis, or a subject belonging to a group known to have a significantly higher than average chance of developing osteoporosis, e.g., post-menopausal women, men over the age of 65, and persons being treated with drugs known to cause osteoporosis as a side effect (such as adrenocorticoid).

By "bone cell activating compound" as used herein is meant a compound which increases the rate of activation of new remodeling units on bone. The concept is described in more detail in Frost, Clinical Orthopedics and Related Research, 143, 227 (1979) and in Rasmussen et al., Metabolic Bone Disease and Related Research, 2, 107 (1980), the disclosures of which are incorporated herein by reference. In most cases this increased rate of activation is initially manifested by an increase in the number of bone resorbing cells and bone resorbing sites. Biochemical indices of skeletal remodeling, such as urinary hydroxyproline levels, are expected to become elevated according to the magnitude of the response to the bone cell activating compound. Specific examples of such compounds are parathyroid hormone (PTH), inorganic phosphate, growth hormone, fluoride, thyroid hormone (e.g. thyroxine), certain vitamin D metabolites and prostaglandins. It may be possible to induce bone cell activation by non-chemical means, e.g. a strict, physical exercise regimen, or electrical currents.

By "bone cell activating amount" as used herein is meant an amount of the bone cell activating agent sufficient to effect a medically significant increase of the rate of activation of new remodeling units. If inorganic phosphate is used as the bone cell activating compound, the amount is in the range of from about 4 mg/kg/day to about 60 mg/kg/day (P.O.) of phosphorus, with amounts of from about 30 mg P/kg/day to about 50 mg P/kg/day preferred. Daily doses of inorganic phosphate should not exceed about 3.6 grams of phosphorous for any subject afflicted with or

at risk to osteoporosis because severe diarrhea and gastrointestinal distress is likely to occur for dosages which exceed this amount.

Bone cell activating amounts of other bone cell activating compounds are as follows: 1,25-dihydroxy vitamin D<sub>3</sub> and other 1-hydroxy vitamin D metabolites: from about 0.001 microgram/kg/day to about 0.03 microgram/kg/day (P.O.); 25-hydroxy vitamin D<sub>3</sub> and other 25-hydroxy vitamin D metabolites (not including 1,25-dihydroxy vitamin D metabolites): from about 0.1 microgram/kg/day to about 3 microgram/kg/day (P.O.); Inorganic fluoride (e.g. sodium fluoride): from about 0.1 mg/kg/day to about 1.0 mg/kg/day F per day (P.O.); thyroxine: from about 0.01 mg/kg/day to about 0.5 mg/kg/day (P.O.); triiodothyroxine: from about 0.1 microgram/kg/day to about 2.5 microgram/kg/day per day (P.O.); prostaglandin PGE<sub>2</sub>: from about 0.1 to about 25 mg/kg/day (P.O.).

The ranges of daily doses of the above mentioned bone cell activating compounds for use in a kit of the present invention are therefore (assuming that the majority of subjects afflicted with or at risk to osteoporosis weigh between about 10 kg and about 100 kg): Inorganic phosphate: from about 0.04 g P to about 3.6 g P (P.O.), from about 0.25 g P to about 3.6 g P (P.O.) preferred, with from about 2 g P to about 3 g P (P.O.) most preferred; 1,25-dihydroxy vitamin D<sub>3</sub> and other 1-hydroxy vitamin D metabolites: from about 0.01 micrograms to about 3 micrograms (P.O.), with from about 0.1 micrograms to about 2 micrograms (P.O.) preferred; 25-hydroxy vitamin D<sub>3</sub> and other 25-hydroxy vitamin D metabolites (not including 1,25-dihydroxy vitamin D metabolites): from about 1 microgram to about 300 micrograms (P.O.), with from about 10 micrograms to about 200 micrograms (P.O.) preferred; Inorganic fluoride (e.g. sodium fluoride): from about 1 mg to about 100 mg (P.O.), with from about 10 mg to about 100 mg (P.O.) preferred; thyroxine: from about 0.1 mg to about 50 mg (P.O.), with from about 1 mg to about 25 mg (P.O.) preferred; triiodothyroxine: from about 1 microgram to about 250 micrograms (P.O.), with from about 10 micrograms to about 150 micrograms (P.O.) preferred; and prostaglandin PGE<sub>2</sub>: from about

1 mg to about 2.5 g (P.O.), with from about 7.5 mg to about 2 g (P.O.) preferred.

By "bone resorption inhibiting polyphosphonate" as used herein is meant a polyphosphonate of the type disclosed in U.S. Patent 3,683,080, granted August 8, 1972, Francis, the disclosures of which are incorporated herein by reference. Preferred polyphosphonates are geminal diphosphonates (also referred to as bis-phosphonates). The polyphosphonates may be administered in the form of the acid, or of a soluble alkali metal salt or alkaline earth metal salt. Hydrolyzable esters of the polyphosphonates are likewise included. Specific examples include ethane-1-hydroxy 1,1-diphosphonic acid, methane diphosphonic acid, pentane-1-hydroxy-1,1-diphosphonic acid, methane dichloro diphosphonic acid, methane hydroxy diphosphonic acid, ethane-1-amino-1,1-diphosphonic acid, ethane-2-amino-1,1-diphosphonic acid, propane-3-amino-1-hydroxy-1,1-diphosphonic acid, propane-N,N-dimethyl-3-amino-1-hydroxy-1,1-diphosphonic acid, propane-3,3-dimethyl-3-amino-1-hydroxy-1,1-diphosphonic acid, phenyl amino methane diphosphonic acid, N,N-dimethylamino methane diphosphonic acid, N(2-hydroxyethyl) amino methane diphosphonic acid, butane-4-amino-1-hydroxy-1,1-diphosphonic acid, pentane-5-amino-1-hydroxy-1,1-diphosphonic acid, hexane-6-amino-1-hydroxy-1,1-diphosphonic acid and pharmaceutically acceptable esters and salts thereof.

The amount of the polyphosphonate to be used is determined entirely by its potency as a bone resorption inhibiting agent. This potency is determined by means of the thyroparathyroidectomized (TPTX) rat model described herein and expressed as the lowest effective dose (LED) of the compound which is defined as the lowest subcutaneously given dose of polyphosphonate, in mg P per kg body weight, which in the TPTX rat model results in an inhibition of the PTH-induced rise in serum calcium level. Since the amount of polyphosphonate to be administered is dependent on the bone resorption inhibition potency of the compound, the amount to be administered is conveniently expressed as multiples of LED. Extrapolation of the dosages for polyphosphonates



from the TPTX rat model to humans is possible based on the observation that oral dosages in humans are proportionally related to the LEDs for polyphosphonates in the TPTX rat model. It is therefore observed that suitable amounts of polyphosphonates for administration in subjects afflicted with or at risk to osteoporosis are from about 0.25 x LED to about 3.3 x LED, while amounts of from about 0.25 x LED to about 2.5 x LED are preferred, and amounts of from 0.50 x LED to 2.0 x LED are most preferred. The LEDs of a number of polyphosphonates are collected in Table I.

Ranges for the daily administration of some polyphosphonates for subjects afflicted with or at risk to osteoporosis are therefore: ethane-1-hydroxy-1,1-diphosphonic acid: from about 0.25 mg P/kg to about 3.3 mg P/kg, with from about 0.25 mg P/kg to about 2.5 mg P/kg preferred; dichloromethane diphosphonic acid: from about 0.12 mg P/kg to about 1.67 mg P/kg, with from about 0.12 mg P/kg to about 1.25 mg P/kg preferred; propane-3-amino-1-hydroxy-1,1-diphosphonic acid: from about 0.025 mg P/kg to about 0.33 mg P/kg, with from about 0.025 mg P/kg to about 0.25 mg P/kg preferred; butane-4-amino-1-hydroxy-1,1-diphosphonic acid: from about 0.0025 mg P/kg to about 0.033 mg P/kg, with from about 0.0025 mg P/kg to about 0.025 mg P/kg preferred; and hexane-6-amino-1-hydroxy-1,1-diphosphonic acid: from about 0.025 mg P/kg to about 0.33 mg P/kg, with from about 0.025 mg P/kg to about 0.25 mg P/kg preferred.

The ranges of daily doses of the above polyphosphonates for use in a kit of the present invention are therefore (assuming that the majority of subjects afflicted with or at risk to osteoporosis weigh between about 10 kg and about 100 kg): ethane-1-hydroxy-1,1-diphosphonic acid: from about 2.5 mg P to about 330 mg P, with from about 2.5 mg P to about 250 mg P preferred, from about 15 mg P to about 200 mg P more preferred, and from about 15 mg P to about 150 mg P most preferred; dichloromethane diphosphonic acid: from about 1.2 mg P to about 167 mg P, with from about 1.2 mg P to about 125 mg P preferred, from about 7 mg P to about 100 mg P more preferred, and from about 7 mg P to about 75 mg P most preferred; propane-3-amino-1-hydroxy-1,1-

diphosphonic acid: from about 0.25 mg P to about 33 mg P, with from about 0.25 mg P to about 25 mg P preferred, from about 1.5 mg P to about 20 mg P more preferred, and from about 1.5 mg P to about 15 mg P most preferred; butane-4-amino-1-hydroxy-1,1-diphosphonic acid: from about 0.025 mg P to about 3.3 mg P, with from about 0.025 mg P to about 2.5 mg P preferred, from about 0.15 mg P to about 2.0 mg P more preferred, and from about 0.15 mg P to about 1.5 mg P most preferred; and hexane-6-amino-1-hydroxy-1,1-diphosphonic acid: from about 0.25 mg P to about 33 mg P, with from about 0.25 mg P to about 25 mg P preferred, from about 1.5 mg P to about 20 mg P more preferred, and from about 1.5 mg P to about 15 mg P most preferred.

By the term "nutrient supplement" as used herein is meant any compound which is generally considered to be a necessary component of a healthy diet and which, in the opinion of the attending physician, is not sufficiently or not consistently ingested by the subject as part of the meals. The term encompasses mixtures of such compounds. The reason why a nutrient supplement is administered to the subject is to make sure that the beneficial results of the regimen are not jeopardized by a poor diet. The most important compounds to be administered as nutrient supplements are therefore those involved in the formation of bone, e.g. vitamin D and calcium.

An important aspect of the present invention is the discovery that too high a dosage of polyphosphonate is detrimental to net bone formation. In fact, dosages which are routinely prescribed for the treatment of Paget's disease appear on the high side for treatment in the present regimen. Generally, polyphosphonate dosage should not exceed about  $3.3 \times \text{LED/day}$ , and are preferably below about  $2.5 \times \text{LED/day}$ . Polyphosphonate dosages below  $2.0 \times \text{LED/day}$  are most preferred.

Neither bone cell activating compounds nor bone resorption inhibiting polyphosphonate are administered during the rest period. This is not to say that no chemicals should be administered to the patient at all during this period. Food supplements like calcium and vitamin D (to be distinguished from bone cell

activating metabolites of vitamin D) can beneficially be administered during this period.

Strict compliance with the above-described regimen is believed to be essential for its success. The kit of the present invention is designed to facilitate such strict compliance in that it contains a means for having the components arranged in a way as to facilitate compliance.

In one specific embodiment of the invention said means is a card having arranged thereupon the components of the treatment regimen in the order of their intended use. An example of such a card is a so-called blister pack. Blister packs are well known in the packaging industry, and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material, covered with a foil of a, preferably transparent, plastic material. During the packaging process, recesses are formed in the plastic foil. The recesses have the size and shape of the tablets or capsules to be packed. Next, the tablets or capsules are placed in the recesses, and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are sealed in the recesses, between the plastic foil and the sheet. Preferably, the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

It is desirable to provide a memory aid on the card, e.g. in the form of numbers next to the tablets or capsules, whereby the numbers correspond with the days of the regimen which the tablets or capsules so specified should be ingested. Another example of such a memory aid is a calendar printed on the card, e.g. as follows "First Week, Monday, Tuesday, ..., etc. ... Second Week, Monday, Tuesday, ...", etc. Other variations of memory aids will be readily apparent. A "daily dose" can be a

single tablet or capsule or several pills or capsules to be taken on a given day. Also, a daily dose of bone cell activating agent can consist of several tablets or capsules, while a daily dose of polyphosphonate is one tablet, or the other way around. The  
5 memory aid should reflect this.

The term "card", as used herein, is not limited to a flat, sheet-like structure. The term includes structures as described above which are folded so as to reduce their planar dimensions; the term further includes a plurality of cards which, combined,  
10 contain the components for the treatment regimen. An example of the latter would be a stack of cards, marked "Week 1", "Week 2", etc., each containing the components of the regimen for one week of treatment. The tablets or capsules may also be arranged on a narrow strip, one after the other; the material of the strip is  
15 preferably flexible, so that it can be wound on a reel. The strip may be perforated so that daily doses can be torn off.

In another specific embodiment of the invention said means is a dispenser designed to dispense said daily doses, one at a time, in the order of their intended use. Preferably, the dispenser is  
20 equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter which indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal  
25 readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

Single-unit dispensers are well known and are being widely used in, e.g., vending machines. The concepts of such machines  
30 are directly suitable for, or easily adaptable to, use in the dispensers of this embodiment of the present invention.

#### Thyroparathyroidectomized (TPTX) Rat Model

To determine the bone resorption inhibition potency of several polyphosphonates, the following animal model was used.

35 In this study 50 male Wistar rats weighing approximately 150-160 grams were thyroparathyroidectomized surgically by the

breeder (Charles River Breeding Laboratories). All rats were double housed on arrival in suspended cages with Purina Laboratory Rodent Chow<sup>R</sup> and tap water ad libitum. After acclimation to the laboratory environment for 3-5 days, the rats were  
5 placed on a low calcium, low phosphorous (.18%/.22%) diet (Teklad<sup>R</sup>) and given 2% (W/V) calcium gluconate supplemented deionized water via water bottles.

On day four of low-calcium diet all rats were anesthetized with Ketaset<sup>R</sup> (Ketamine Hydrochloride, 100 mg/ml, Bristol  
10 Myers), 0.10 ml/100 grams of body weight, weighed and then bled orbitally for serum total calcium analysis using Flame Atomic Absorption (FAA). All rats weighing less than 180 grams were eliminated from the study. Animals were then randomized statistically such that the mean total calcium for each group was the  
15 same. Only rats deemed hypocalcemic (total calcium  $\leq$  8.0 mg/dl) were placed in study groups (6 animals in each group).

Treatments with the various experimental compounds commenced on day 6 and lasted through day 9 of the study (at 1:00 P.M. each day). Dose solutions were prepared to be given at a  
20 constant rate of 0.2 ml/100 grams of body weight subcutaneously in the skin flap where the hind leg meets the torso. All rats were weighed and dosed daily. A 25 gauge 5/8" needle was used to administer drug, alternating dose sites daily. On day 9 all rats were fasted in the afternoon at approximately 4:00 P.M. On  
25 day 10 of study no treatment was given. In the morning a 600  $\mu$ l sample of whole blood was collected from each rat in Microtainer (B-D#5060) serum separator tubes for serum total calcium (FAA). Two 125  $\mu$ l samples of heparinized whole blood were also collected to be used for ionized calcium analysis (radiometer ICA1). Im-  
30 mediately following blood collection all rats were weighed and injected with bovine parathyroid hormone subcutaneously at a rate of 75 USP (filtered) per 100 grams of body weight. Blood sampling for total and ionized calcium was repeated three hours post-PTH injection.

Statistics

All pre- and post-PTH total and ionized calciums were statistically analyzed for significance compared to PTH alone (control) using Student's t-test, analysis of variance, and their non-parametric equivalents. The post minus pre-change and % change were also determined on calcium levels and pre-drug vs post-drug body weights.

Materials

Low calcium and phosphorous diets used were prepared by Teklad<sup>R</sup> Test Diets (Harlan Industries, Madison, Wisconsin 53711; Order #TD82195) in a pellet form of approximately .18% calcium and .22% phosphorous. The diets contained all the essential vitamins and minerals required for the rat, with the exception of calcium and phosphorous. The calcium and phosphorous levels of the pellets were verified analytically.

All dosing solutions of compounds to be tested for bone resorption inhibition potency were adjusted to pH 7.4 with sodium hydroxide and prepared in 0.9% saline (Abbott NDC 0074-1583-03), Abbott Labs, North Chicago, IL 60064, USA). Dosing solution concentrations were adjusted to a dosing rate of 0.20 ml/100 grams of body weight.

PTH was acquired as a powdered bovine extract (Sigma Chemical Co., P.O. Box 14508, St. Louis, Missouri, order #P-0892, Lot #72F-9650) at an activity of 138 USP units per mg. PTH was prepared in 0.9% saline such that the final concentration was 100 U.S.P./ml. All solutions were filtered through a #40 Whatman Filter Paper then .45  $\mu$ m Metrical<sup>R</sup> filter.

The physiological effect of the PTH challenge is a rise in serum calcium level. Since the animals were on a low calcium diet, an observed increase in serum calcium level is the result of a resorption of bone material. Since polyphosphonates tend to inhibit resorption of bone material, the animals pretreated with polyphosphonate showed a rise in serum calcium level upon PTH challenge which was less than that found in control animals which had been treated with saline vehicle instead. The lowest dose at which the polyphosphonate is capable of inhibiting bone resorp-

tion, as evidenced by a decreased rise in serum calcium upon PTH challenge, is a measure of the bone resorption inhibition potency of the polyphosphonate. Where necessary the test was repeated, whereby the animals were administered with .5 and .2 x LED, in order to refine the determination of LED. The LED values of some representative diphosphonates are presented in Table I.

TABLE I

Lowest Effective (antiresorptive) Dose (LED) Values

Compound *	LED (mg P/kg)
ethane-1-hydroxy 1,1-diphosphonic acid (EHDP)	1.0
dichloromethane diphosphonic acid (Cl <sub>2</sub> MDP)	0.5
propane-3-amino-1-hydroxy-1,1-diphosphonic acid (APD)	0.10
butane-4-amino-1-hydroxy-1,1-diphosphonic acid (ABDP)	0.01
hexane-6-amino-1-hydroxy-1,1-diphosphonic acid (AHDP)	0.10

\*) All compounds were in aqueous solution, the pH of which had been adjusted to 7.4 with NaOH. At this pH the diphosphonic acids are present as their disodium salts.

Clinical Test

Five patients clinically diagnosed as suffering from osteoporosis were subjected to a treatment regimen according to the present invention as follows. Each patient was subjected to from 3 to 8 cycles, each cycle consisting of (a) a bone activating period of 3 days during which 2 tablets of Phosphate Sandoz<sup>TM</sup> were administered 3 times daily (each tablet contains 500 mg of elemental phosphorus); (b) a bone resorption inhibition period of 14 days during which the patients received 5 mg/kg/day (corresponding to 1.24 mg P/kg/day, or 1.24 x LED) of DIDRONEL (Norwich Eaton Pharmaceuticals, Norwich, N.Y.) divided into 3 doses (each DIDRONEL tablet contains 200 mg of disodium EHDP); (c) a rest period of 73 days during which the patients received a diet which was verified by dieticians to contain a minimum of 1 g/day of calcium. Each patient was examined clinically before the following cycle was instituted. All patients were subjected to a standardized double tetracycline labeled transillac crest bone biopsy before starting the treatment and after 3, 6 or 8 cycles were completed. The bone biopsy is described in detail by

Melsen et al., "The Role of Bone Biopsy In the Diagnosis of Metabolic Bone Disease," Orthop. Clin. of NA, 12, 571-602 (1981), the disclosures of which are incorporated herein by reference. All bone biopsies were prestained with osteochrome stain as described by Villanueva in "Theory and Practice of Histotechnology," 2nd Ed., C.V. Mosley Co., London, 1980, pp. 100-101, the disclosures of which are incorporated herein by reference. The biopsies were then embedded in methacrylate as described by Anderson in "Manual for the Examination of Bone," CRC Press, 1982, pp. 27-29, the disclosures of which are incorporated herein by reference. Twenty sections were cut on a Jung K heavy duty Microtome, ten sections of each biopsy were stained with toluidine blue and ten sections remained unstained for viewing with ultraviolet light. Histomorphometric analyses of static and dynamic parameters of trabecular bone were carried out twice by two individuals on each biopsy using a Zeiss Photomicroscope III with the necessary attachment to use the osteoplan semi-automatic method as described by Malluche et al., Calcif. Tissues, Int., 1982, 34, 439-448, the disclosures of which are incorporated herein by reference.

All patients reported subjective improvement in their symptomatology and a marked increase in their daily physical activity with less periods spent at forced rest because of pain. The patient who had been subjected to 3 cycles of therapy showed a directional improvement in trabecular bone mass. The three patients who had been subjected to 6 cycles and the one patient who had been subjected to 8 cycles showed a dramatic improvement in their trabecular bone mass, their trabecular diameter, as well as a dramatic improvement in the dynamic assessment of their trabecular bone remodeling activity as measured by histomorphometric analyses of transillac crest biopsies. These results indicate that there was a significant improvement in the osteoporotic condition of these patients.

Other osteoporotic patients were subjected to a similar regimen, except that the daily dosage of disodium EHDP during the bone resorption inhibition period was 15 mg/kg/day (3.72 x LED).



No significant increase of bone mass was observed. These results indicate that the treatment regimen of the present invention results in a dramatic increase of trabecular bone mass, but that a daily dosing of polyphosphonic acid in excess of  $3.3 \times \text{LED}$  is counterproductive.

The treatment regimen is varied as indicated in Table II.

TABLE II

Bone Activating Period			Bone Resorption Inhibition Period			Rest Period	Total Cycle
Days	Compound	Dose/day	Days	Compound	Dose/day (mg P/kg)	Days	Days
1	1,25-Vit.D <sup>(a)</sup>	1 ug	10	Cl <sub>2</sub> MDP	2.5	30	41
3	NaF	20 mg	12	APD	0.5	40	55
5	PTH 1-34 <sup>(b)</sup>	100 ug	17	AHDP	0.03	50	72
4	PGE <sub>2</sub> <sup>(c)</sup>	10 mg/kg	20	ABDP	0.005	80	104

(a) 1,25 dihydroxy vitamin D<sub>3</sub>

(b) parathyroid hormone 1-34

(c) prostaglandin E<sub>2</sub>

A treatment regimen consisting of one or more of the above cycles results in an appreciable alleviation of osteoporotic conditions.

## EXAMPLE I

A kit for use in a regimen for treatment or prevention of osteoporosis is made up as follows:

A slip case 4-3/4" wide x 8-1/2" high x 5-1/2" deep (about 12 cm x 21-1/2 cm x 14 cm) contains 10 cards (blister packs) of 4-3/4" x 8-1/2" (about 12 cm x 21-1/2 cm). The first card has arranged thereupon 3 rows of 6 tablets each. Each tablet contains inorganic phosphate, 500 mg phosphorus per tablet. The rows are marked (from left to right) "Day 1", "Day 2", and "Day 3" and the patient is instructed to take two tablets three times per day (i.e., the total daily dose consists of 6 tablets of 500 mg P each).

The second card contains 14 tablets, each tablet containing 400 mg EHDP (DIDRONEL), Norwich Eaton Pharmaceuticals, Norwich, NY). The tablets are arranged in 4 rows of 3 tablets each and a 5th row of 2 tablets. Printed on the card, next to each tablet, are the words "Day 4", "Day 5",...etc. through "Day 17".

The remaining 8 cards each contain 20 capsules, each capsule containing 300 mg of calcium and 200 units of vitamin D. Printed on each card are rectangular boxes, such that each box contains two capsules (i.e., 10 boxes per card; one daily dose is two capsules, each of which contains 300 mg of calcium and 200 units of vitamin D). The boxes are marked "Day 18", "Day 19", etc., through "Day 97" on the last card.

The last card further contains a printed reminder that a renewal prescription should be obtained.

Similar kits are put together wherein the 3 daily doses of inorganic phosphate are replaced with daily doses of other bone cell activating compounds. For example: 5 daily doses of 0.5 microgram each of 1,25-dihydroxy vitamin D<sub>3</sub>; 2 daily doses of 100 micrograms each of 25-hydroxy vitamin D<sub>3</sub>; 4 daily doses of 80 mg F each of inorganic fluoride; 1 daily dose of 20 mg thyroxine; 3 daily doses 70 micrograms each of triiodothyroxine; or 4 daily doses of 50 mg each of prostaglandin PGE<sub>2</sub>.

Similar kits are further made by replacing the 14 daily doses of sodium ethane-1-hydroxy-1,1-diphosphonate ("EHDP") with daily doses of other bone resorption inhibiting polyphosphonates. For example: 10 daily doses of 20 mg P each of dichloromethane diphosphonic acid; 18 daily doses of 8 mg P each of propane-3-amino-1-hydroxy-1,1-diphosphonic acid; 20 daily doses of 0.7 mg P each of butane-4-amino-1-hydroxy-1,1-diphosphonic acid; or 15 daily doses of 10 mg P each of hexane-6-amino-1-hydroxy-1,1-diphosphonic acid.

Similar kits are further made by replacing the 80 daily doses of calcium and vitamin D with, e.g., 60 daily doses of 500 mg each of calcium; 40 daily doses of 400 units each of vitamin D; or 30 daily doses of one placebo tablet each.

WHAT IS CLAIMED IS:

CLAIMS

1. A kit for use in a regimen for treatment or prevention of osteoporosis, said regimen comprising sequential administration of a bone cell activating compound, a bone resorption inhibiting polyphosphonate, and a placebo, or a nutrient supplement, said kit characterized in that it contains the following components:

- (a) from 1 to 5 daily doses of a bone cell activating amount each of a bone cell activating compound;
- (b) from 10 to 20 daily doses of from 0.25 x LED to 3.3 x LED each of a bone resorption inhibiting polyphosphonate; and
- (c) from 30 to 100 daily doses of a placebo or a nutrient supplement;

and a means for having the components arranged in a way as to facilitate compliance with the regimen.

2. The kit of Claim 1 characterized in that the daily doses of the bone resorption inhibiting polyphosphonate are from 0.25 x LED to 2.5 x LED.

3. The kit of Claims 1 or 2 characterized in that said means is a card having arranged thereupon said components in the order of their intended use.

4. The kit of Claims 1 or 2 characterized in that said means is a dispenser designed to dispense said daily doses, one at a time, in the order of their intended use.

5. The kit according to any of Claims 1-4 characterized in that the bone cell activating compound is an inorganic phosphate and each daily dose is from 250 mg P to 3.6 g P, preferably from 2 g P to 3 g P.

6. The kit according to any of Claims 1-4 characterized in that the bone cell activating compound is selected from 1,25-dihydroxy vitamin D<sub>3</sub> and each daily dose is from 0.1 microgram

to 2 micrograms; 25-hydroxy vitamin D<sub>3</sub> and each daily dose is from 10 micrograms to 200 micrograms; an inorganic fluoride and each daily dose is from 10 mg F to 100 mg F; thyroxine and each daily dose is from 1 mg to 25 mg; triiodothyroxine and each daily dose is from 10 micrograms to 150 micrograms; and prostaglandin PGE<sub>2</sub> and each daily dose is from 7.5 mg to 2 g.

7. The kit according to any of Claims 1 and 3-6 characterized in that the bone resorption inhibiting polyphosphonat is selected from ethane-1-hydroxy-1,1-diphosphonic acid, or a pharmaceutically acceptable salt or ester thereof, and each daily dose is from 15 mg P to 200 mg P, preferably from 15 mg P to 150 mg P; dichloromethane diphosphonic acid, or a pharmaceutically acceptable salt or ester thereof, and each daily dose is from 7 mg P to 100 mg P; preferably from 7 mg P to 75 mg P; propane-3-amino-1-hydroxy-1,1-diphosphonic acid, or a pharmaceutically acceptable salt or ester thereof, and each daily dose is from 1.5 mg P to 20 mg P, preferably from 1.5 mg P to 15 mg P; butane-4-amino-1-hydroxy-1,1-diphosphonic acid, or a pharmaceutically acceptable salt or ester thereof, and each daily dose is from 0.15 mg P to 2.0 mg P, preferably from 0.15 mg P to 1.5 mg P; and hexane-6-amino-1-hydroxy-1,1-diphosphonic acid, or a pharmaceutically acceptable salt or ester thereof, and each daily dose is from 1.5 mg P to 20 mg P, preferably from 1.5 mg P to 15 mg P.

8. A kit for use in a regimen for treatment or prevention of osteoporosis, said regimen comprising, sequential administration of inorganic phosphate, ethane-1-hydroxy-1,1-diphosphonic acid or a pharmaceutically acceptable salt thereof, and a mixture of vitamin D and calcium, said kit characterized in that it contains the following components:

- (a) a card having arranged thereupon 3 daily doses of inorganic phosphate, each daily dose containing 2500 mg P;
- (b) a card having arranged thereupon 14 daily doses of 100 mg P each of ethane-1-hydroxy-1,1-diphosphonic acid,

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- 3 -

or a pharmaceutically acceptable salt or ester thereof;

- (c) a plurality of cards containing 80 daily doses, each dose containing 700 mg calcium and 400 units of vitamin

D.

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# EUROPEAN SEARCH REPORT

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EP 85 20 0650

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,A	US-A-4 230 700 (MARION D. FRANCIS) * Column 11, line 65 - column 13, line 37 *	1-8	A 61 K 31/66 A 61 K 33/42 A 61 K 31/59
D,A	--- US-A-4 330 537 (MARION D. FRANCIS) * Column 11, line 58 - column 13, line 19 *	1-8	
D,A	--- US-A-3 683 080 (MARION D. FRANCIS) * Column 13, line 64 - column 14, line 21 *	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 61 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 01-08-1985	Examiner BRINKMANN C.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	